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Noise effect in metabolic networks*

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Constraint-based models such as flux balance analysis (FBA) are a powerful tool to study biological metabolic networks. Under the hypothesis that cells operate at an optimal growth rate as the result of evolution and natural selection, this model successfully predicts most cellular behaviours in growth rate. However, the model ignores the fact that cells can change their cellular metabolic states during evolution, leaving optimal metabolic states unstable. Here, we consider all the cellular processes that change metabolic states into a single term 'noise', and assume that cells change metabolic states by randomly walking in feasible solution space. By simulating a state of a cell randomly walking in the constrained solution space of metabolic networks, we found that in a noisy environment cells in optimal states tend to travel away from these points. On considering the competition between the noise effect and the growth effect in cell evolution, we found that there exists a trade-off between these two effects. As a result, the population of the cells contains different cellular metabolic states, and the population growth rate is at suboptimal states.

Keywords: noise, metabolic networks, flux balance analysis
PACC: 8700, 8725

1. Introduction

Complete sequences of the genome combined with stoichiometric information about biochemical reactions for several model organisms allow us to reconstruct metabolic networks on the genome-scale level.[1,2] Among these models, constraint-based models such as flux balance analysis (FBA)[3−6] have been well developed to interpret the properties of metabolic networks and predict cellular behaviours. The metabolic flux distributions, growth rate, and by-product production provided by FBA are in good agreement with experiments.[7]

The flux balance analysis is based on a hypothesis that bacteria maximize their growth rate after long-term evolution under natural selection.[8,9] This hypothesis is reasonable from the point of view of Darwinian evolution, because for a single cell organism, the growth rate is the most important evolutionary driving force. However, FBA contains certain shortcomings. The first problem is the degeneracy of the optimal states. Recently, Mahadevan *et al* found that a genome-scale network has multiple flux distributions with the same optimal value of growth rate.[10] This means that biological systems have a large range of variability among the optimal solutions; interpreting the behaviour of metabolic networks using a single feasible solution from optimization is not enough to understand all possible flux patterns. A current trend in solving this problem is flux variability analysis (FVA),[11] which measures the possible range of each reaction instead of interpreting a specific feasible solution. The second problem is the noise effect. The optimal growth hypothesis does not pay much attention to the stability of optimal states and the capability of cells maintaining optimal growth under perturbations. Because of the stochastic properties of biochemical reactions, regulatory circuits, genetic mutations and so forth, metabolic networks face large fluctuations. These fluctuations will surely influence the behaviour of cells; they are not negligible in the prediction of cell growth. Currently, there are also debates on the hypothesis of using only cellular growth as the objective function.[12,13]

The feasible solution space in FBA, in which the fluxes meet all the physiological and thermodynamic constraints, includes all the allowable metabolic flux distributions. It determines the metabolic capacity of the system[14] and the robustness of the network. Recent studies have shown that feasible solution space is biologically meaningful, not just an artificial side-effect of lacking sufficient knowledge on genetic regulation and other factors.[15,16] For each specific cell growth rate, there is a corresponding solution space. The larger the size of the solution space for a specific growth rate, the more alternative states that cells can adopt to maintain the current growth rate under

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perturbations.[17] In this sense the size of the solution space for a particular growth rate reflects the stability of cells under this growth rate.

Fluctuations in gene transcription regulation, gene mutation, and biochemical reactions in metabolic networks can endanger the stability of the optimal growth state, which usually corresponds to a very small solution space. To cope with these fluctuations, a population of cells may operate at a more stable configuration. This configuration includes various suboptimal manners, each occupying a different solution space. This reflects heterogeneity in metabolic states and phenotypes. Heterogeneous metabolic states, especially growth rates, would cause fitness variations and growth competition in the same culture. As a result, certain dominant modes of metabolic states would be selected in a cell population. The structure of the cell population is the focus of this study. Recently, the experiments of Fong *et al* have shown that multiple biologically meaningful flux states are active in different conditions;[8] great variations in gene expression levels are also detected between genetically identical cells taken from the same culture.[18,19] All these observations support this line of thinking.

In order to study the extent to which the noise effect influences metabolic behaviours, we integrated all factors causing fluctuations in dynamics of metabolic networks into a single term 'noise' and studied the noise effect on the final structure of cell population. By proposing an ergodic hypothesis and simulating a state of a cell randomly walking in solution space, we constructed an evolutionary model to explore the competition between noise effect and growth effect in cell evolution. We found that there exists a trade-off between these two effects. As a result, the population of the cells contains different cellular metabolic states, and the population growth rate is at suboptimal states. Turning off the noise, the cell culture will gradually travel to the optimal states, as the FBA model predicts.[3−6]

2. The ergodic hypothesis on cell state movement in solution space

In a cell population, variations in cell growth rate have been observed in different experiments[20−22] even when the population was genetically homogeneous. It was confirmed that fluctuations in concentrations of metabolites, metabolic reaction rates, and activities of some pathways are the major causes of these heterogeneous metabolic behaviours.[18,19] For example, some molecules that control genetic regulatory circuits operate at extremely low concentrations (tens or hundreds of molecules per cell), which gives large fluctuations in enzyme concentrations; biochemical reactions with low reaction rates lead to asynchronism in genetic transcriptions; transcriptional switch circuits and mutations during gene duplications yield stochastic properties. All these sources of noise influence the dynamics of metabolic networks and the flux distribution of the network. But a quantitative study of the details of these fluctuation impacts on metabolic networks is very difficult. To bypass this difficulty, we simplified all the biological processes that change cellular metabolic states into a single term 'noise', and assume that without growth competition and selection pressure, all the metabolic states in the solution space of FBA should be equally treated. Thus under the actions of various noises, a cell at one metabolic state can travel to any other state in the solution space with equal probability. We call this assumption the ergodic hypothesis for metabolic networks. Integrating all biological processes into the single term 'noise' sacrifices the determinate cellular regulation mechanism in metabolic networks. However, this simplified assumption may help us to understand essential mechanisms of the metabolic network, such as the stability of optimal metabolic states and the way that the metabolic network reacts to 'noise'.

Solution space in FBA is a closed high-dimensional polyhedron containing points which represent all flux distributions satisfying stoichiometric, thermodynamic and physiological constraints. Our previous study using expanded flux variability analysis[17] demonstrated that for a given specific cell growth rate, there is a corresponding solution subspace; the size of the subspace decreases when the growth rate increases. That means the subsection of solution space corresponding to the highest growth rate is much smaller than other ones. Ergodic random walks allow that metabolic networks can achieve any state represented by a point in solution space with the same probability, thus without growth competition, phase points have more possibility of walking into subsections with low growth rates.

To quantitatively apply the ergodic hypothesis, we use a hit-and-run algorithm[23] to simulate phase points randomly walking in solution space (see Appendix for detailed information). The sequence of points generated can distribute unitarily in the solution space if the step number of random walks is large enough, so this is a reasonable algorithm to simulate random walks of phase points and satisfies the ergodic hypothesis proposed for metabolic networks. With a fixed initial growth rate, we first generate a series of phase points in solution space with this initial growth rate, then these points are allowed to randomly walk in a hit-and-run manner. After a fixed random walk step, we record the growth rates of all of these phase points and obtain the distribution of final growth rates. This distribution measures the transition probability of cells from the initial growth rate to other growth rates.

With enough samples, we study the distribution function on growth rates of all sample phase points after fixed steps (t) of random walks. Figure 1 provides two typical case studies. One observes that the distribution on growth rate after specific random walk steps t is a Gaussian function (Figs.1(a1) and (b1)). Two parameters can be extracted from these distributions: the average growth rate $g(t)$ and standard deviation $\theta(t)$ around $g(t)$. We found that as t increases, $g(t)$ decreases almost linearly no matter whether cells initially operate at optimal growth states ($g_0 = 1.553$, Fig.1(a)) or suboptimal growth states ($g_0 = 0.95$, Fig.1(b)). Meanwhile, $\theta(t)$ satisfies the power law as a function of t; the exponents are different with different initial growth rates. The same results were also observed for other initial growth states (data not shown here).

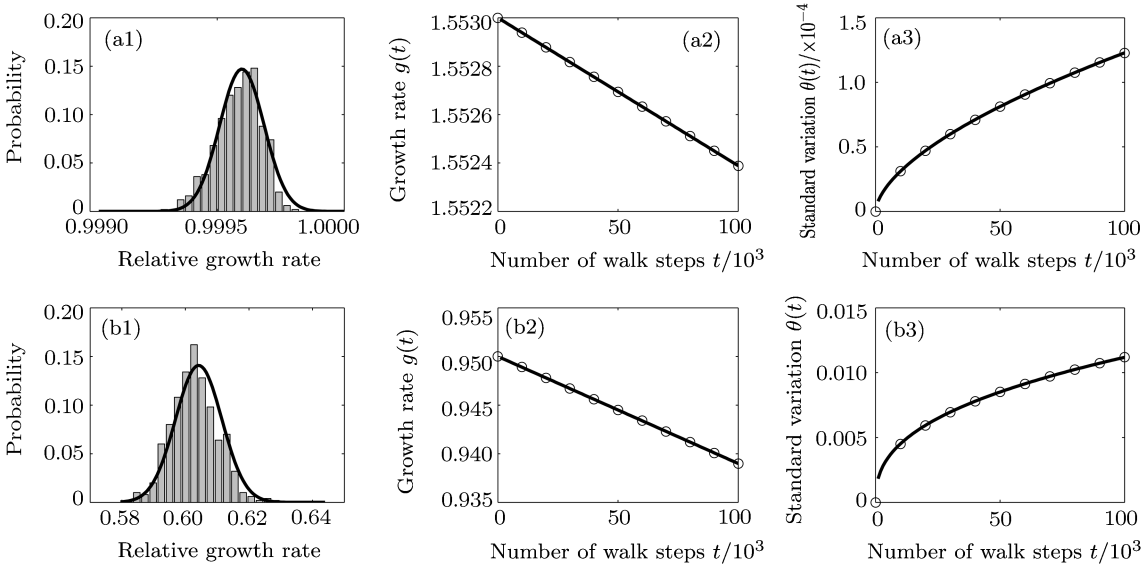


Fig.1. Simulation of random walks within solution space. (a1) Distribution of phase points initializing from optimal states on growth rate after $t = 10^5 (= 100\times10^3)$ steps of random walks. The bars are simulation results from 500 samples with the same initial growth rate, optimal growth rate $g_0 = 1.553$; the solid lines represent Gaussian functions fitted to the post-transition phase point distribution. (a2) Mean growth rate $g(t)$ change during random walks. (a3) Standard variation of the growth rate $\theta(t)$ change during random walks. (b1) Growth rate change of another 500 samples with initial growth rate, suboptimal growth rate $g_0 = 0.95$. (b2) and (b3) The same as (a2) and (a3) respectively. The circles are obtained through simulation, the solid lines are from fitting.

The results of the random walk simulations allow us to extract quantitative information on phase point travelling. We notice that $g(t)$ as a function of t measures phase points drifting towards areas with lower growth rates, and $\theta(t)$ describes the diffusion of phase points. Thus, we define drift velocity $V(G)$, diffusion exponent $\gamma(G)$ and diffusion coefficient $D(G)$ for each initial growth rate ($g(0)$) in solution space: $\Delta g(t) = g(t) - g(0) = V(g(0))t$, and $\theta(t) = D(g(0))t^{\gamma(g(0))}$. Figure 2 presents our calculation results of these three parameters as a function of initial growth rate. In general, these three parameters are all functions of initial phase point $g(0)$; they reflect the geometric properties of the area around the initial point in solution space.

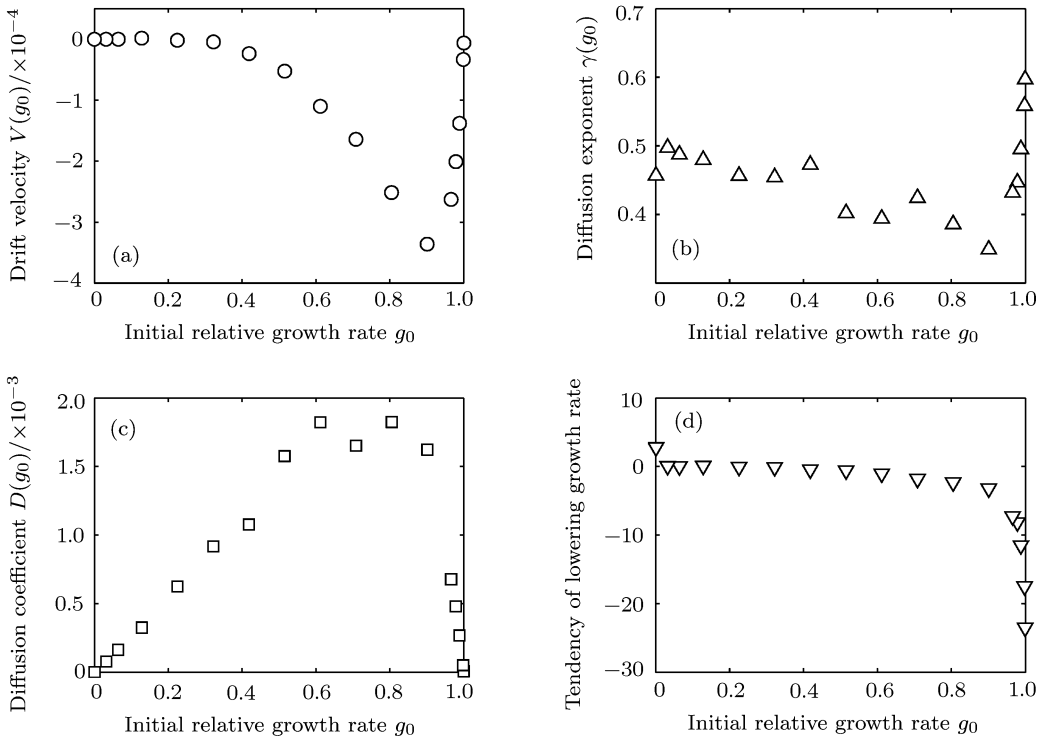


Fig.2. Parameters under different initial growth rates g_0. (a) Drift velocity during random walks $V(g_0)$. For all initial growth rates, drift velocity V is lower than 0. (b) Diffusion exponent $\gamma(g_0)$. (c) Diffusion coefficient $D(g_0)$. (d) Parameter which measures the tendency of lowering growth rate during random walks $(\mathrm{d}g(t)/\mathrm{d}t)/(\mathrm{d}\theta(t)/\mathrm{d}t)$; here we set $t = 100 \times 10^3$. Phase points with higher initial growth rates have a higher tendency to lower growth rates.

One of the geometric properties of solution space is constraints in the metabolic system. In the hit-and-run algorithm, the maximum possible step length is restricted by constraints in metabolic networks. If some fluxes are constrained in a narrow range, the step length will be doomed to have a small value. This situation happens when the cellular growth rate is near zero and near the optimal value. Given an inner point in such areas of solution space, it should not go too far, otherwise it will violate constraints, which are represented by a series of inequalities (for details see the Appendix). This property determines the near zero drift velocity and near zero diffusivity when the growth rate is near zero and at the maximum growth rate (Figs.2(a) and (c)).

Another geometric property of solution space is that the volume of solution space with a higher growth rate is smaller than that with a lower growth rate, which is consistent with previous results.[17] Since every step of the random walk is based on a stochastically chosen direction and step length, a phase point will have more probability of travelling to areas with a lower growth rate, so that drift velocity is always lower than zero (Fig.2(a)). We use $(\mathrm{d}g/\mathrm{d}t)/(\mathrm{d}\theta/\mathrm{d}t)$ to measure the tendency of this movement. Although both drift velocity and diffusion velocity are influenced by constraints, their quotient represents this tendency. As shown in Fig.2(d), the tendency drops almost exponentially as the initial growth rate decreases, especially when phase points are near optimal states. The geometric property about the relative size of solution space indicates that noise tends to damage cellular fitness and drives metabolic systems away from optimal states. From these points of view, without growth competition, the states of maximum growth rate are not stable against perturbations.

3. Adaptive evolution model with noise effect

In the previous section, we showed that if growth competition and evolution selection are not considered, noise in metabolic networks leads to a phase point in solution space travelling to states with lower growth rates. The situation changes dramatically when the growth competition is considered, because as all cells keep growing exponentially, the relative

advantage of individuals with high growth rates over those with low growth rates is also exponential. This exponential advantage for cells with high growth rates should be balanced by the trend of cells lowering their growth rate under noise. Meanwhile the trend of lowering growth rate measured by the quotient of drift velocity and mean diffusion velocity also increases exponentially near the maximum growth rate (see Fig.2(d)). Thus the noise effect will be strong enough to balance the growth competition advantage within a population.

We use an adaptive evolution model to estimate the influence of both of these two effects on the behaviours of metabolic networks. The adaptive evolution model is constructed to simulate the relevant experimental process. In the model, cells in a population are divided into a series of subgroups on the basis of their growth rate, and an n-dimensional vector $P = \{p_1, p_2, \ldots, p_n\}$ is defined to record populations in each of the n subgroups. To simplify the calculation process, we assume that all cells keep exponential growth because of a dilution process in the experimental design,[8] which avoids cells being overcrowded and reaching static phase. Notice that dilution does not change the population distribution of cells on growth rates. Growth competition can be achieved by allowing cells in subgroup i to grow with their own growth rate x_i. To reflect the noise effect in metabolic networks, we define a transition probability matrix to describe the cells in subgroup i travelling to subgroup j. The formula of the transition probability from subpopulation j to subpopulation i is determined by random walk simulations. It takes a Gaussian form:

$$\mathrm{Prob}_{ij} = \begin{cases} \dfrac{A}{\sqrt{2\pi\theta_j(t)}} \exp\left[-\dfrac{(x_i - x_j + |\Delta g_j(t)|)^2}{2\theta_j(t)^2}\right], & i \neq j, \\ 1 - \sum_{k\neq j} \mathrm{Prob}_{kj}, & i = j. \end{cases} \tag{1}$$

Here $|\Delta g_j(t)|$ measures the growth rate decrease of cells in subgroup j within a generation; $\theta_j(t)$ measures the diffusion of phase points from an initial growth rate x_j. We can measure $|\Delta g_j(t)|$ and $\theta_j(t)$ for some specific initial growth rates because the drift velocity (V_j), the diffusion coefficient (D_j) and the diffusion exponent (γ_j) can be obtained directly from random walk simulations; those for other initial growth rates can be obtained through interpolation. So both $\Delta g_j(t)$ and $\theta_j(t)$ can be determined solely by t, which shows how many steps of random walks occur in a generation. Another important parameter A represents the portion of the whole population that jumps between different metabolic states because of noise; this parameter measures the noise level. Integrating both noise and growth factors, the dynamic growth equation for subgroup i can be written as follows:

$$\frac{\mathrm{d}p_i}{\mathrm{d}t} = x_i p_i + \sum_j \mathrm{Prob}_{ij} p_j - \sum_j \mathrm{Prob}_{ji} p_i. \tag{2}$$

Notice that the above equations are linear; we can write them in the form of an n-dimensional vector and a matrix,

$$\frac{\mathrm{d}\mathcal{P}}{\mathrm{d}t} = \mathcal{M}\mathcal{P}. \tag{3}$$

Without the noise effect $A = 0$, the transition probability $\mathrm{Prob}_{ij} = 0$. One can easily find that the only mode of optimal growth states will be selected after growth competition and evolution. This is consistent with the FBA prediction. In the presence of noise, a trade-off must be achieved between the noise effect and growth effect in the evolution. As a result, a final steady state can be expected for this linear model. The average growth rate of evolved culture G_s is the largest eigenvalue of matrix $\mathcal{M}$. A steady population distribution on growth rate P_s is the eigenvector responding to the largest eigenvalue G_s of $\mathcal{M}$. We can connect these simulation results to experimental results.

4. Comparison of simulation results with experiment

The trade-off between growth rate and biomass yields has been explored theoretically and experimentally.[20,24] The experimental data from Novak[20] for population distribution on growth rate can be used to justify our adaptive evolution model. The experiments measure sample growth rates in four Escherichia coli cultures, and show that the population distribution $P(g)$ on growth rate in each population is a single peak function. The histograms in Fig.3 show the experimental results. Notice that the growth rate of cells in a population varies around a single, suboptimal growth rate.

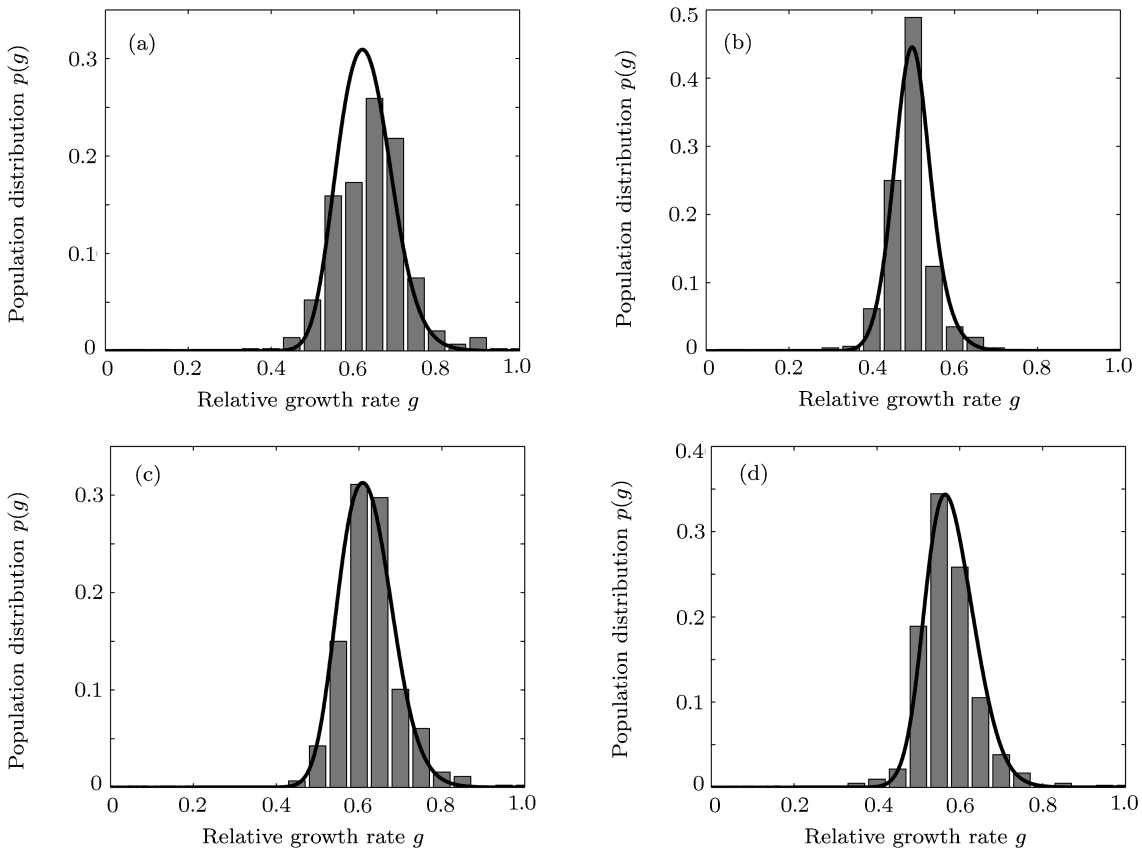


Fig.3. Post-evolution population distribution on growth rate. The bars represent population distribution on growth rate from experiments;[20] figures 3(a)–3(d) represent four cellular populations in the experiment. The solid lines represent population distribution $P(g)$ from the adaptive evolution model; we set $t = 90\times10^3$ for all of these populations, A =0.38 (a); 0.51 (b); 0.39 (c); 0.43 (d).

In order to fit our adaptive evolution model with the experimental data, we need to find suitable parameters in Eq.(1). There are two free parameters in the model: t, which reflects the travelling times in each generation of cells; A, which reflects the noise level of the evolution system. In the simulation, we found that a single peak distribution can be achieved in the model only when t is limited within a small range between (80–200)$\times10^3$, otherwise additional peaks can be generated. This means that in some cases evolution can lead to growth state divergence and multiple growth rates. We notice that although the parameter t greatly influences the number of peaks in the model, it has only a small effect on the width and position of the main peak when it can maintain the single peak growth rate distribution.

The parameter A determines the scale of noise. In the calculation, we found that the parameter A determines both the suboptimal growth rate (the mean value in Fig.3) and the growth rate variation within a population (the variation in Fig.3). So we only need to adjust the value of A in order to fit both the average growth rate and peak width observed in the experiments well. A typical noise source comes from the random partition of metabolites and enzymes during cell division. In the extreme case, we consider that cells in the division phase take extensive random walks in solution space, while in other phases the noise effect is not as intensive. Based on this argument, we can estimate a lower limit of noise level A in a cell generation. For Escherichia coli, cell division takes 20 min per generation, which lasts for less than one hour. Considering other sources of noise like genetic mutation, we expect that on average A is not less than 1/3.

The solid line in Fig.3 is our experimental fitting. From our computation, we find that our model can fit experimental data well with a reasonable choice of parameters. The value of parameter t is chosen to be 90×10^3, in the range of (80–120)$\times10^3$, to guarantee the single peak requirement. Meanwhile, parameter A is set to different values for the four experimental

populations, ranging from 0.38–0.51. All of them are larger than 1/3, reflecting the noise that commonly exists in cellular populations.

5. Discussion

In this study, we investigated the noise effect on the behaviour of metabolic networks. We assume that various biological processes in metabolic networks, no matter whether stochastic or determinate, can be treated as noise, which drives phase points to randomly travel in solution space. Under the influence of the noise, cells have a tendency to lower their growth rate because the solution space volume for low growth rates is much larger than that for high growth rates. The noise effect also causes phenotype diversity in a cell culture. This point of view is supported by experimental observations. Furthermore, the qualitative consistency between our simulation and experimental result justifies our strategy in calculating cell population.

In our model, we constructed a semi-quantitative method to qualitatively show how metabolic networks behave differently when noise is considered. If a more accurate and quantitative model is required, one needs to add more constraints from genetical regulation into the model in order to simulate the determinate process and incorporate more biological information to shape solution space, because the shape of the solution space will greatly influence the accuracy of our results. In our current model, the constraints of the solution space come mainly from mass conservation and thermodynamic laws, whereas the effects of genetic regulation mechanisms are only considered as part of the noise, which leads to ergodic behaviours. In fact, genetic regulations can largely reshape the solution space. One of the current trends in FBA is to incorporate genetic regulation rules as constraints into the optimization process.[3,25] For example, Palsson *et al* proposed a method to integrate regulatory information in metabolic flux analysis.[26−28] They applied global Boolean regulation networks[28,29] as constraints to reduce feasible solution space. But one has to obtain flux distributions on the basis of the optimal growth hypothesis to control variables in regulation networks. The SRFBA[30] provides us with a promising alternative method. The self-consistent Boolean states of regulation networks would exclude some reactions from the active reaction list, so greatly reducing the size of the solution space. Although currently there are technical difficulties in incorporating regulation rules into FBA models, it can be expected that the ideal strategy to predict cellular growth rate should incorporate all the known genetic regulations in a metabolic network, evaluating the solution space, and using our method to calculate the structure of the cell phenotype in growth.

Finally, we try to compare the competition between the noise effect and the growth effect in cell evolution with competition between entropy and energy in thermodynamics. A trade-off between energy reduction and entropy increase leads to a free energy minimum. Similar results can be seen in metabolic systems. Growth rate functions are similar to energy and the evolutionary volume response to a specific growth rate seems like entropy. Cells maintain a balance between these two factors to take advantage of both growth and robustness, thus they have to grow at suboptimal states.

Appendix

(i) Flux balance analysis

The flux balance analysis (FBA) model includes all metabolic reactions and metabolites. The metabolic reconstruction begins from an annotated genome sequence by identifying enzymatic reactions relevant to these annotated genes. Other reactions are also added to close the mass balance based on physiological and biochemical data, as well as transport mechanisms.

The assumption of a steady state of concentrations of the intracellular metabolites requires a mass balance of all the internal metabolites; the sum of all influxes equals the sum of all outflows

$$\sum_{i=1}^{n} S_{ji} v_i = 0, \tag{A1}$$

where S_{ji} is an element of the $m \times n$ stoichiometric matrix S, m and n being the number of metabolites and the number of reactions respectively; v_i is an element in the net reaction rate vector V. Usually S is a singular matrix so equation (A1) has multiple solutions.

Constraints currently considered in FBA mainly come from thermodynamic laws and physiological rules. The feasible solution space being thus defined is a high-dimensional polyhedron. In detail, solutions are subject to two constraints: the nutrition (i.e., carbon, oxygen, etc.) uptake constraint specified by:

$$v_i^{\text{uptake}} \leq v_i^{\text{uptake,max}} \tag{A2}$$

and the thermodynamic constraint specified by:

$$v_i \geq 0. \tag{A3}$$

Traditional FBA seeks the maximum growth rate in Eq.(A1) under these constraints.

Variation of a particular reaction measures the feasible range of this reaction.

$$\delta_i = v_{\max,i} - v_{\min,i}. \tag{A4}$$

By computing variations of reactions we found that some fluxes are blocked and this means that constraints in this model have greatly reduced the degree of freedom. Among all 1084 fluxes (some are artificially defined for convenience of computation and analysis)[1] in metabolic networks, 444 of them are blocked and only have zero flux variability when growing in glucose substrate. This means that biochemical reactions related to these fluxes are inactivated during the steady state assumed in FBA. Null space of the the stoichiometric matrix which excludes column vectors responding to blocked reactions is a 334-dimensional infinite linear space when constraints are not considered; however, after constraints are exposed on metabolic networks, we can only get a 208-dimensional solution space. Results from random walk simulations reveal some geometric properties of this 208-dimensional solution space.

(ii) The hit-and-run algorithm

The algorithm includes the following steps. Consider the bounded solution space $S \subset \mathcal{R}_n$:

Step 0 Find an inner point representing specific growth rate $G_0 \in \mathcal{R}$ in the solution space. Set $i = 0$.

Step 1 Generate a direction d_i from the unitary possibility distribution over the possible direction set D. Find the set $\Lambda_i = \{\lambda \in \mathcal{R} | G_i + \lambda d_i \in S\}$.

Step 2 Generate a real number λ_i from a unitary distribution over the set Λ_i.

Step 3 Set $G_{i+1} = G_i + \lambda_i d_i$ and set $i = i + 1$. Go to Step 1.

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